Received: 9 September 2025 | Accepted: 23 October 2025

DOI: 10.1111/eci.70147

NARRATIVE REVIEW

WILEY

Integrated biomarker landscape for the early detection and management of calcific aortic valve disease

Alberto Cook-Calvete[1] | **Silvia Moreta**[1] | **Maria Delgado-Marin**[1] | **Blanca Fernandez-Rodriguez**[1] | **Carlos Zaragoza**[2,3] | **Marta Saura**[1,3,4]

[1]Departamento de Biología de Sistemas Facultad de Medicina, Universidad de Alcalá, Alcalá de Henares, Spain

[2]Unidad Mixta de Investigación Cardiovascular Universidad Francisco de Vitoria Hospital Ramón y Cajal (IRYCIS), Madrid, Spain

[3]Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[4]Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

Correspondence
Marta Saura, Departamento de Biología de Sistemas Facultad de Medicina, Universidad de Alcalá, 28871 Alcalá de Henares, Spain.
Email: marta.saura@uah.es

Abstract

Background: Calcific aortic valve disease (CAVD) is the predominant valvular pathology in older adults, advancing from aortic sclerosis to life-threatening stenosis. Without effective medical therapies, intervention mainly relies on timely valve replacement, although silent myocardial and valvular damage may progress before symptoms arise. Early, non-invasive detection of disease activity is a crucial unmet need.

Aims: To review circulating and mechanistic biomarkers reflecting the core pathogenic pathways of CAVD and asses their potential for early detection and patient-specific risk stratification.

Methods: Narrative review of literature focusing on traditional protein biomarkers, emerging non-coding RNAs, and extracellular vesicles (EVs) associated with lipid oxidation and inflammation, bone and mineral metabolism, extracellular matrix (ECM) remodelling, endothelial dysfunction and non-coding RNA regulation.

Results: Traditional protein biomarkers—such as lipoprotein(a), osteopontin, fetuin-A, galectin-3 and matrix metalloproteinases—offer insights into the disease and correlate with disease burden but lack sensitivity for detecting early-stage CAVD. Emerging non-coding RNA markers, including long non-coding RNAs (lncRNAs) and microRNAs (like miR-30b and miR-125b), show promise as predictive and diagnostic tools by mediating key molecular pathways involved in calcification and inflammation. EVs, which carry proteins, lipids and nucleic acids across all pathogenic pathways, provide stable and comprehensive signatures that enhance risk stratification compared to conventional markers. Notably, no single biomarker has demonstrated sufficient sensitivity or specificity across all stages of the disease. Combining proteins, RNAs and EV cargo into integrative, multimodal panels—supported by proteomics and transcriptomics—provides the greatest potential for early detection and patient-specific management. However,

further validation in prospective cohorts and standardization of assays are necessary before clinical implementation.

Conclusion: Biomarker-guided approaches could revolutionize CAVD management by enabling early detection and patient stratification before irreversible valvular damage occurs.

KEYWORDS

aging, biomarkers, calcific aortic valve disease, cardiovascular disease

1 | INTRODUCTION

Calcific aortic valve disease (CAVD) is a progressive, chronic condition characterized by fibrotic changes and calcification of the aortic valve leaflets, ultimately leading to aortic stenosis (AS). CAVD is among the most prevalent valvular heart diseases in older populations, with prevalence increasing markedly with age: approximately 2% of individuals over 65, 3% at 75 and 4% at 85 exhibit clinical aortic stenosis, representing the advanced stage of CAVD.[1,2]

Clinically, CAVD progresses through distinct but overlapping stages. The initial stage, aortic valve sclerosis, involves leaflet thickening without significant obstruction to blood flow. Over 5–10 years, the disease advances to leaflet stiffening, reduced valve opening and increased left ventricular pressure overload, eventually causing heart failure and higher mortality. Once severe AS develops, ventricular compensation declines, symptoms appear and prognosis worsens significantly.[3] Despite its prevalence, no pharmacological therapy has proven effective in halting or reversing CAVD progression, leaving surgical or transcatheter valve replacement as the only definitive treatment for advanced stages.

Early identification of affected individuals is critical, as myocardial damage may remain reversible in presymptomatic stages. While valve replacement is indicated for symptomatic severe AS due to high mortality risk, management of asymptomatic patients is challenging. These patients may appear clinically stable yet harbour silent myocardial injury and remain vulnerable to sudden deterioration.[4] Myocardial fibrosis, often present before symptoms, underscores the need for tools capable of detecting disease activity prior to overt decompensation.[5,6] A deeper understanding of the transition from valvular sclerosis to stenosis is essential, as early intervention may significantly alter disease progression.

CAVD is now recognized as an active, regulated process rather than a passive degenerative phenomenon of aging. Its progression reflects a complex interplay between mechanical stress, valve endothelial dysfunction, lipid deposition, chronic inflammation, osteogenic reprogramming of valvular interstitial cells (VICs) and extracellular matrix (ECM) remodelling.[7] Disease commonly initiates on the aortic side of the valve, where oscillatory shear stress and endothelial injury compromise the integrity of valve endothelial cells (VECs), promoting lipid infiltration and immune cell recruitment. In response to mechanical and inflammatory stimuli, VECs may undergo endothelial-to-mesenchymal transition, acquiring a mesenchymal and profibrotic phenotype that contributes to early ECM remodelling and VIC activation.[8] This phenotypic plasticity enhances the local inflammatory environment, favouring the nucleation of calcific nodules.[9] Monocytes, macrophages and T cells infiltrate the subendothelial layer, releasing cytokines and proteolytic enzymes that drive VIC differentiation toward osteoblast-like cells.[1] Together, these processes sustain a self-perpetuating cycle of inflammation and mineralization underlying progressive CAVD.

Current diagnostic tools rely primarily on echocardiography and computed tomography, which detect only advanced calcification and structural changes.[10] Given the unmet need for effective therapies to slow or reverse CAVD progression, the identification of novel biomarkers, whether circulating, genetic, or mechanistic, may improve early detection, refine risk stratification and optimize intervention timing.[11,12] Beyond their diagnostic value, biomarkers can provide insights into underlying pathophysiology and support personalized monitoring strategies that balance procedural risk with disease trajectory.[13,14]

This review highlights recent advances in both established and emerging biomarkers of CAVD, examining their biological relevance and exploring their potential clinical applications for early detection, improved pathophysiological understanding and therapeutic monitoring.

2 | PATHOPHYSIOLOGICAL AXES REFLECTED BY CIRCULATING AND MOLECULAR BIOMARKERS IN CALCIFIC AORTIC VALVE DISEASE

2.1 | Endothelial dysfunction and inflammation

Endothelial dysfunction represents a pivotal initiating event in the pathogenesis of CAVD, driving a cascade of molecular and cellular changes that progressively remodel the aortic valve.[15] The aortic side of the leaflet, chronically exposed to oscillatory and low shear stress, exhibits increased susceptibility to endothelial injury and lipid infiltration compared to the ventricular side, which is protected by stable laminar flow.[16,17] Studies using porcine and human aortic valve endothelial cells have shown that disturbed flow activates pro-inflammatory transcriptional programs through NF-κB (a pro-inflammatory transcription factor) and MAPK signalling, leading to the upregulation of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), as well as reduced expression of endothelial nitric oxide synthase (eNOS).[18] These changes impair nitric oxide bioavailability, favouring oxidative stress and establish a pro-inflammatory microenvironment conducive to monocyte recruitment and immune cell adhesion on the valvular surface.

Once endothelial integrity is compromised, circulating lipoproteins can infiltrate the subendothelial matrix, triggering early pathological remodelling.[19] Histological and proteomic analyses of human aortic valves removed at various stages of disease have shown early accumulation of apolipoprotein B, oxidized LDL and lipoprotein (a) [Lp(a)], highlighting their role in the early development of CAVD.[20] Lp(a) carries oxidized phospholipids that activate Toll-like receptor 2 and the lectin-like oxidized LDL receptor on endothelial and interstitial cells, stimulating secretion of interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1).[21] These mediators recruit macrophages and intensify local inflammation, while macrophages and T lymphocytes release additional pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interferon-gamma (IFN-γ), promoting osteogenic differentiation of VICs.[15,21] Studies with primary human VICs have shown that exposure to TNF-α or IL-6 elevates osteogenic transcription factors, including RUNX2 and Osterix, and enhances alkaline phosphatase (ALP) activity, thereby promoting calcium deposition.[15] Oxidized LDL further enhances expression of bone morphogenetic proteins 2 and 4 (BMP-2 and BMP-4), supporting osteogenic pathways via the SMAD1/5/8 signalling cascade.[21,22]

Chronic inflammation links early endothelial dysfunction with fibrotic and calcific remodelling of the valve and adjacent myocardium.[23] Innate and adaptive immune responses are activated within the valvular microenvironment, involving macrophages, T lymphocytes and mast cells that release a broad spectrum of cytokines and growth factors, including IL-1β, IL-6, TNF-α, IFN-γ and TGF-β.[24] These mediators sustain chronic inflammation and promote ECM remodelling. Indeed, transcriptomic and single-cell RNA sequencing analyses have identified the upregulation of pro-inflammatory pathways, including NF-κB, JAK/STAT and the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, in diseased human valves.[25] Activation of NLRP3 in resident macrophages and VICs promotes IL-1β secretion, thereby amplifying fibroblast activation and osteogenic differentiation.[26] Meanwhile, TGF-β signalling through the SMAD2/3 cascade drives excessive matrix deposition and myofibroblast transition, contributing to leaflet thickening and increased valvular stiffness. Persistent inflammatory activity may extend beyond the valve, fostering interstitial myocardial fibrosis and left ventricular remodelling, as demonstrated by imaging and histopathological studies.[27,28]

A further layer of complexity arises from the crosstalk between VECs and VICs. Under pro-inflammatory or mechanical stress, VECs can undergo endothelial-to-mesenchymal transition, acquiring a myofibroblastic phenotype that directly contributes to the development of fibrosis and calcification.[29] This process has been shown both in vitro and in murine models, where TGF-β1 and BMP-2 signalling promotes mesenchymal transition by downregulating VE-cadherin and upregulating α-smooth muscle actin and vimentin in VECs.[18] Importantly, mutations in NOTCH1, identified in families with bicuspid aortic valve and early-onset calcification, lead to reduced inhibition of the BMP2 pathway and enhanced osteogenic activity,[30,31] thereby establishing a genetic link between developmental signalling and degenerative calcification.[32] As the disease advances, chronic inflammation and oxidative stress create a self-sustaining cycle of tissue remodelling.[33] Under this scenario, activated macrophages secrete matrix metalloproteinases (MMP-2, MMP-9) and cathepsins that degrade ECM components, releasing bioactive fragments that further stimulate VIC proliferation and differentiation.[34,35] Concurrently, pro-calcific factors such as bone sialoprotein, osteocalcin and osteopontin (OPN) accumulate within the valvular ECM, while matrix vesicles released from VICs serve as nucleation sites for calcium phosphate crystal deposition.[36–38]

Finally, the pathophysiological overlap between CAVD and coronary artery disease provides an important critical clinical perspective.[39] Both entities share key mechanisms, including endothelial dysfunction, lipid accumulation, oxidative stress and chronic inflammation, yet diverge in their cellular and structural outcomes.[40,41] Early CAVD lesions resemble atherosclerotic plaques, exhibiting lipid deposition and macrophage infiltration.[42,43] Imaging F^{-18}-NaF PET studies have further revealed concurrent microcalcification activity in the valve and coronary arteries, suggesting a systemic procalcific inflammatory milieu.[41] However, as osteogenic differentiation becomes predominant, valve disease progression increasingly dissociates from circulating lipid levels, which may explain the lack of therapeutic benefit of statin administration in these patients.[44] Notably, elevated levels of Lp(a) and OxPL are independently associated with accelerated progression of both aortic valve and coronary calcification.[45,46] Hence, CAVD and coronary artery disease should be viewed as interconnected manifestations of a shared inflammatory and osteogenic vascular disease, modulated by local hemodynamic and cellular contexts.[47]

2.2 | Extracellular matrix remodelling and calcium deposition

ECM remodelling is a central component of the fibrocalcific progression of CAVD, linking chronic inflammation to mechanical stiffening of the valve leaflets. Fibrotic remodelling is orchestrated by profibrotic cytokines such as TGF-β and insulin-like growth factor-1 (IGF-1), which stimulate VICs to differentiate into myofibroblast-like cells, characterized by increased α-SMA expression and secretion of collagen types I and III.[1,7] The chronic activation of these pathways leads to excessive collagen cross-linking and loss of valve elasticity, a hallmark of the fibrotic phenotype preceding calcification. Matrix metalloproteinases (MMPs) play a dual role in this process. MMP-1, MMP-2 and MMP-9 are key components of collagen degradation and ECM turnover.[1] Finally, in the advanced stages of calcific aortic valve disease (CAVD), significant calcium buildup and bone-like mineralization occur, leading to stiffened and dysfunctional valve structures. This bone formation within the valve tissue is believed to result from the further transformation of VICs from myofibroblasts into osteoblast-like cells, which promote valve calcification through mechanisms resembling those of normal bone development.

3 | CIRCULATING AND MOLECULAR BIOMARKERS IN CALCIFIC AORTIC VALVE DISEASE: AN INTEGRATED PERSPECTIVE

The identification of circulating and molecular biomarkers in CAVD offers a dynamic window into the underlying pathophysiological processes that drive disease onset and progression, as well as their relationship with disease severity. Recent advances in proteomics, metabolomics and transcriptomics have uncovered novel candidates, including non-coding RNAs, matrix remodelling proteins and calcification-related enzymes, that may reflect early subclinical pathological changes highlighting the potential of these biomarkers for early diagnosis and risk stratification.[1] In parallel, newly developed PET- and CT-based imaging biomarkers are now being explored to quantify valve inflammation and active calcification in vivo.[48]

3.1 | Lipid- and inflammation-related markers

Lipid metabolism and inflammation play pivotal roles in the onset and progression of CAVD. Among the lipid-related biomarkers, Lp(a) remains the most robustly validated, acting through its OxPL cargo and apolipoprotein(a) kringle IV type 10 domain, which confer strong pro-inflammatory and pro-thrombotic properties.[49] Currently, Lp(a) is the only biomarker used to help identify individuals with a genetic predisposition and a higher risk for CAVD.[50] Elevated Lp(a) concentrations are associated with a faster onset and progression of disease, particularly during the early and subclinical stages of aortic sclerosis, characterized by valve thickening without significant obstruction.[51] However, this association weakens in advanced symptomatic aortic stenosis, where calcification is mainly driven by the existing calcium load rather than Lp(a) levels.[52] The influence of Lp(a) may also depend on molecular interactions—for instance, complexes with apolipoprotein C-III appear more relevant to moderate stages of disease—and may vary across ethnic groups, with weaker or weaker associations reported in Hispanic and Asian populations.[53] Large population-based studies and Mendelian randomization analyses have established a causal association between genetically elevated Lp(a) and incident AS, positioning Lp(a) as one of the most clinically relevant biomarkers identified to date.[14] Mechanistically, Lp(a)-derived OxPL stimulates VICs to adopt an osteogenic phenotype through upregulation of BMP-2, RUNX2 and ALP, thereby promoting calcification independently of traditional lipid levels.[54]

TABLE 1 Biomarker classes and their usefulness across stages of calcific aortic valve disease.

Biomarker class	Examples	Pathophysiological role	Stage	Clinical usefulness
Lipoprotein(a)	Lp(a)	Genetic driver of early calcification (autotaxin–LPA signaling)	Early	Strongest genetic biomarker; stable quantification via ELISA
Oxidized Phospholipids	OxPLs	Reflect oxidative stress and microcalcification activity	Early–Mid	Correlate with F-18-NaF PET; lipidomic assays non-standardized
Endothelial/ Inflammatory markers	VCAM-1, CXCL12, KLKB1	Indicate endothelial activation and immune recruitment	Early	Detect subclinical inflammatory activity; sensitive but nonspecific
EV-derived markers	Annexins, OPN, MMP-9, GDF-15	Intercellular signaling and early calcification	Mid	High predictive value; EV isolation not standardized
Osteogenic proteins	BMP-2, ALP, OPG	Mediate mineralization and osteoblastic differentiation	Late	Reliable ELISA quantification; reflect structural progression
Fibrotic remodeling proteins	Galectin-3, MMP-28	Mirror extracellular matrix turnover and fibrosis	Mid–Late	Indicate fibrotic remodeling; limited disease specificity

Note: The table summarizes key circulating and molecular biomarkers, indicating their predominant biological roles, expression patterns across disease stages and clinical or technical applicability. Early-stage biomarkers reflect potentially reversible processes such as endothelial dysfunction and inflammation, whereas late-stage biomarkers indicate irreversible changes, including osteogenic differentiation and calcification. The stage color gradient goes from green to red, representing disease progression from early (green) to late (red) stages, passing through yellow, black, and orange.

Abbreviations: ALP, alkaline phosphatase; BMP-2, bone morphogenetic protein-2; CXCL12, CXC motif chemokine 12 or stromal cell-derived factor 1 (SDF-1); GDF-15, growth differentiation factor-15; ICAM-1, intercellular adhesion molecule-1; KLKB1, plasma kallikrein, kininogenase B1; Lp(a), lipoprotein(a); MMP-9, MMP-1 and MMP-28, matrix metalloproteinase-9, -1, -28; OPG, osteoprotegerin; OPN, osteopontin; OPN, osteopontin; OxPLs, lipid oxidation products; VCAM-1, vascular cell adhesion molecule-1.

Despite these nuances, Lp(a) remains a promising biomarker and therapeutic target. However, its limited capacity to predict disease progression suggests that its primary value lies as a valuable diagnostic biomarker of disease.[7,38,55]

As illustrated in Table 1, integrating lipidomic and inflammatory biomarkers with imaging markers of active calcification may enable more precise stratification of patients at risk of rapid disease progression and provide molecular endpoints for forthcoming Lp(a)-lowering trials. Besides Lp(a), recent proteomic and transcriptomic analyses have identified inflammatory chemokines that reflect and possibly mediate disease activity. Elevated circulating CXCL12 and CXCL6 (C-X-C chemokine ligand-12 and -6) have been linked to increased immune cell infiltration within the valve and enhanced recruitment of monocyte-derived macrophages, which secrete IL-6, TNF-α and MMPs—key drivers of fibro-calcific remodelling.[56] Similarly, plasma kallikrein B1 (KLKB1), an inflammatory protease detected by high-throughput plasma proteomics, has also emerged as a marker of early valvular inflammation and endothelial dysfunction.[56] Taken together, these findings suggest that systemic inflammation, as reflected in circulating chemokine profiles, parallels histological inflammatory activity within the valve and hence could refine molecular phenotyping of early disease.

Adipokines, particularly leptin, further bridge metabolic dysregulation and valvular inflammation. Leptin promotes osteogenic differentiation of VICs by activating the JAK/STAT and MAPK pathways and correlates with aortic valve calcification burden assessed by CT imaging.[56] In experimental models, leptin enhances oxidative stress and TGF-β signalling, amplifying the profibrotic response, whereas leptin receptor deficiency attenuates calcification and matrix remodelling.[57] Nonetheless, the clinical interpretation of leptin levels must consider confounders such as obesity, insulin resistance and systemic inflammation, which often coexist in patients with CAVD.[58]

3.2 | Endothelial dysfunction and nitric oxide-related biomarkers

Endothelial dysfunction represents an early and persistent driver of CAVD pathogenesis, serving as a bridge between hemodynamic stress, inflammation and osteogenic

activation. Under physiological conditions, VECs inhibit calcification by producing NO, a function dependent on endothelial nitric oxide synthase activity. However, chronic exposure to oxidative stress, the oxidation of eNOS cofactor BH4 and disturbed laminar flow disrupt NOS enzymatic activity, leading to decreased NO bioavailability and increased production of reactive oxygen species (ROS).[1] This imbalance promotes VIC activation, proliferation and differentiation into osteoblast-like cells by upregulating NOTCH1, NF-κB and BMP-2 signalling pathways.[31,59] Emerging data highlight the role of integrin-linked kinase (ILK) as a key regulator of eNOS phosphorylation and endothelial homeostasis. Reduced ILK expression in VECs has been linked to increased valvular calcification severity underscoring its potential as both a mechanistic mediator and a biomarker of endothelial dysfunction in CAVD.[60] Experimental silencing of ILK in cultured VECs recapitulates key pathogenic features: reduced NO production, increased oxidative stress and induction of osteogenic transcription factors, confirming its causal involvement.[61,62] Moreover, endothelial injury enhances the release of circulating endothelial EVs (CD144+/CD31+), which mirror valvular stress and may serve as early indicators of endothelial dysfunction and disease activity.[62]

Overall, the endothelial–NO axis plays a pivotal role in the early pathogenesis of CAVD, with ILK emerging as a key mediator of endothelial dysfunction.[60] Although its intracellular localization limits direct applicability as a circulating biomarker, the identification and validation of accessible surrogates such as exosomal ILK,[63] ILK-regulatory microRNAs (miRNAs), or downstream signalling readouts could enable early detection and refined risk stratification in patients at preclinical or progressive stages of the disease.[64]

3.3 | Extracellular matrix remodelling biomarkers

Extracellular matrix (ECM) remodelling plays a key role in the fibrocalcific progression of the disease leading to calcium deposition and valve stiffening. Matrix metalloproteinases (MMPs), play an important role in the remodelling of the ECM. In particular, MMP-1 degrades type I and III collagen, which are major structural components of the fibrosa layer, thereby directly contributing to matrix remodelling. Studies have shown that MMP-1 expression is significantly elevated in patients with aortic valve stenosis compared with control subjects. Notably, serum MMP-1 levels peak in moderate stages of the disease and decline in severe stenosis, reflecting a transition from active inflammation to predominant calcification, suggesting that MMP-1 is a candidate marker for early disease diagnosis.[65,66]

Recently, MMP-28 (also known as epilysin) has also emerged as a novel candidate for CAVD. Elevated plasma levels of MMP-28 have been reported in patients with coronary artery disease, reinforcing its association with atherosclerosis, a condition that shares pathophysiological mechanisms with CAVD.[67] Consistent with these findings, Zhou et al. reported elevated plasma MMP-28 concentrations not only in patients with mild-to-moderate aortic stenosis but also in those with severe disease, where levels were even higher.[68] These increases correlated positively with hemodynamic severity, including higher mean transvalvular pressure gradients and greater peak aortic valve flow velocities.[68] However, important gaps remain before MMP-28 can be fully considered a reliable diagnostic tool for CAVD. To date, no direct mechanistic evidence links MMP-28 activity to valvular calcification or fibrosis, and it remains uncertain whether its elevation may act as a causal mediator or merely reflects a secondary response to tissue injury. Therefore, although MMP-28 shows potential as a biomarker of disease severity and progression, further mechanistic and longitudinal studies are required to validate its functional role and prognostic value in CAVD.

Beyond the role of MMPs in valvular calcification, the Galectin family of proteins, especially Galectin-3 (Gal-3), has gained recognition as key mediators of fibro-inflammatory signalling in both cardiac and valvular tissues.[69] Gal-3 binds β-galactoside residues on cell surface glycoproteins, promoting fibroblast proliferation, collagen synthesis and macrophage recruitment.[70] Clinically, elevated plasma Gal-3 correlates with aortic stenosis severity and faster progression of valve sclerosis, and its predictive value extends to myocardial fibrosis and adverse cardiac remodelling.[70] However, Gal-3 is elevated in many fibrotic/inflammatory cardiovascular conditions, reducing its specificity for CAVD. Many clinical studies also show confounding by renal function (Gal-3 levels correlate negatively with eGFR), which may limit its specificity in risk stratification.[71]

Together, these ECM-related biomarkers, MMPs and Gal-3, reflect the balance between matrix degradation and fibrogenesis, serving as dynamic indicators of active disease processes in CAVD as reflected in Table 1.

3.4 | Bone- and mineral-metabolism-related biomarkers

The process of valvular calcification in CAVD closely mirrors osteogenic bone formation, characterized by the activation of osteogenic signalling pathways in VICs. Key

osteogenic mediators, including bone BMP-2, OPN and osteoprotegerin (OPG), are consistently overexpressed in calcified aortic valves, reflecting the reprogramming of VICs toward an osteoblast-like phenotype.[72] BMP-2 acts as a potent inducer of RUNX2, the master transcription factor for osteogenic differentiation, thereby promoting the expression of alkaline phosphatase and matrix vesicle release, which facilitates calcium phosphate crystal deposition.[73] In vitro and ex vivo studies demonstrate that BMP-2 expression is upregulated by oxidative stress, Lp(a)-derived oxidized phospholipids and inflammatory cytokines such as TNF-α and IL-6, linking the inflammatory milieu to osteogenic signalling.[74] Among matrix proteins, OPN serves as both a structural and regulatory component. Although initially expressed as an inhibitor of calcification, persistent inflammation and mechanical stress transform OPN into a marker of active mineralization. Circulating OPN levels correlate with early calcification burden and faster hemodynamic progression of aortic stenosis.[75] Conversely, elevated circulating levels of OPG, a decoy receptor for receptor activator of nuclear factor κB ligand, have been associated with adverse outcomes and increased cardiovascular mortality, indicating that OPG may serve as a marker of systemic cardiovascular stress rather than valve-specific pathology.[76]

Systemic inhibitors of ectopic mineralization also modulate the biology of CAVD. Fetuin-A is a liver-derived glycoprotein that binds calcium–phosphate complexes and prevents hydroxyapatite precipitation. Low Fetuin-A concentrations are associated with increased valvular calcium load and greater stiffness, especially in individuals with chronic kidney disease or diabetes.[77,78] Experimental models demonstrate that Fetuin-A deficiency accelerates valvular and vascular calcification by disrupting its inhibitory interaction with the TGF-β/BMP pathway and reducing the clearance of calciprotein particles.[79] However, despite its clear mechanistic relevance, the clinical utility of this biomarker remains limited, given significant interindividual variability and a strong influence from systemic inflammatory and metabolic states.[78]

3.5 | Long non-coding RNAs and microRNAs as early predictors and biomarkers

Non-coding RNAs (ncRNAs), including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), have emerged as potent regulators of gene expression and promising non-invasive biomarkers in CAVD. LncRNAs modulate key pathogenic processes: osteogenic differentiation, inflammation, endothelial dysfunction and ECM remodelling, through complex post-transcriptional and epigenetic mechanisms,[80,81] but in addition, lncRNAs also serve as critical modulators of osteogenic signalling. Examples include H19, one of the most extensively studied lncRNAs, which promotes VICs' osteogenic transformation by interacting with miR-675 and modulating Wnt/β-catenin and RUNX2 pathways.[82] The lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) regulates inflammatory and profibrotic gene networks, while TUG1 and SNHG3 act as 'molecular sponges' for osteogenesis-inhibiting miRNAs, thereby facilitating calcification.[83,84] Notably, lncRNA FGD5-AS1 is downregulated in CAVD and modulates osteogenic differentiation via the miR-497-5p/BIRC5 regulatory pathway, thereby increasing ALP activity. Moreover, intraperitoneal administration of FGD5-AS1 in the ApoE mouse model of atherosclerosis, significantly attenuated valvular leaflet thickening and calcium deposition.[85] On the other hand, transcriptomic/profiling studies (bulk RNA-seq, microarrays) have, in addition, identified panels of differentially expressed lncRNAs in CAVD vs. control valves, providing potential candidates for biomarker testing.[86] However, very few have been validated as reliable circulating biomarkers in human cohorts yet.[82]

Dysregulation of specific miRNAs contributes to multiple disease mechanisms. miR-30b, miR-139-5p, miR-145-5p and miR-125b are downregulated in CAVD and typically suppress RUNX2, SMAD1 and other osteogenic transcription factors.[87–90] Their loss facilitates VICs' differentiation into osteoblast-like cells and enhances local inflammation. Differential expression of miR-21, miR-30b, miR-133, miR-155 and the miR-143/145 cluster has been documented in both valvular tissue and circulating plasma from CAVD patients, suggesting that these miRNAs may reflect disease activity. In particular, downregulation of miR-30b and miR-133 is associated with osteogenic differentiation of VICs, whereas the upregulation of miR-21 promotes fibrosis and calcification.[91]

Circulating and extracellular vesicle-associated miRNAs remain stable in plasma, making them attractive biomarkers of early disease and risk stratification.[92] When combined with clinical parameters, comprehensive miRNA profiling enhances the ability to predict rapid valvular calcification and may inform the development of timely therapeutic interventions. To this regard, integrative miRNA panels, when combined with echocardiographic and clinical parameters, have shown improved predictive accuracy for rapid calcification progression and adverse outcomes.[93] For instance, the study by Fabiani et al. demonstrated that higher levels of miR-29, miR-133 and miR-1 were associated with a more advanced stage of aortic stenosis, typically characterized

by a reduced ejection fraction. Conversely, miR-21 and miR-29 result in independent predictors of reverse remodelling and systolic function recovery after aortic valve replacement.

Together, lncRNAs and miRNAs may represent the next generation of precision biomarkers and potential therapeutic regulators, bridging molecular insights with clinical translation in CAVD.[81] Nevertheless, challenges remain in assay standardization, inter-patient variability and validation in large, longitudinal cohorts.

Complementing the potential of nucleic acids, circulating proteomic profiles have also provided valuable insight into systemic alterations accompanying CAVD.[94] High-throughput assays, such as Olink and SomaScan, have identified panels of proteins associated with disease severity and progression, including MMP-12, C1QTNF1, GDF-15 and soluble Suppression of Tumorigenicity 2 markers reflecting ECM turnover, inflammatory stress and tissue remodelling. Integrating proteomic profiles with RNA-based signatures, along with clinical and imaging parameters, may enable the development of composite biomarker panels capable of predicting disease progression and therapeutic response with greater accuracy.[14]

3.6 | Extracellular vesicles: Integrative platforms in calcific aortic valve disease

EVs have become key mediators of intercellular communication and promising next-generation biomarkers for CAVD. These nanosized, membrane-bound vesicles, released by VECs, VICs and infiltrating immune populations, encapsulate proteins, lipids and RNAs reflective of the cellular state from which they originate.[95] Within the calcifying valve, EVs are not passive byproducts but active participants in disease propagation: their cargo includes bone morphogenetic proteins (BMP-2, BMP-4), annexins and phosphatidylserine-rich membranes that serve as nucleation sites for calcium-phosphate deposition, thereby linking molecular signalling to the physical formation of microcalcifications.[96,97] Unlike soluble biomarkers, EVs preserve this molecular information within their lipid bilayer, allowing remarkable stability in biofluids such as plasma, urine and saliva.[97] This feature provides a unique advantage for non-invasive sampling and temporal monitoring of disease dynamics.

Comprehensive proteomic and transcriptomic profiling of circulating EVs has revealed distinct molecular signatures associated with CAVD. Valve-derived EVs are enriched not only in osteogenic mediators (BMP-2, annexin A6) but also in OxPLs and miRNAs, notably downregulated miR-30b and upregulated miR-122-5p and miR-125b, which reflect and perpetuate calcification dynamics.[88,98,99] EVs can also transport regulatory miRNAs that fine-tune gene expression in target cells. Notably, Goody et al. demonstrated that EVs derived from AS patients exhibit elevated levels of miR-145-5p, which modulates the ZEB2–ALPL signalling axis and directly enhances VICs' calcification.[89] Moreover, EVs transport fibrotic and inflammatory mediators including TGF-β, Galectin-3 and MMP-9, thereby reflecting the principal pathogenic axes of CAVD: lipid oxidation, inflammation, endothelial dysfunction, ECM remodelling and also cellular senescence.[100,101] Proteomic analyses have identified vesicular annexins, alkaline ALP and ectonucleotide pyrophosphatase/phosphodiesterase-1 as nucleation factors driving calcium-phosphate deposition.[102]

This multifaceted composition makes EVs 'molecular integrators' of the valvular microenvironment, capturing signals from multiple cellular sources simultaneously. Indeed, both EV concentration and cargo composition have been shown to correlate with hemodynamic severity, histological calcification burden and disease progression, as depicted in Figure 1, which summarizes the role of EVs as carriers of the main molecular pathways and representative biomarkers involved in CAVD.[98,103]

Compared with other markers, EV-associated biomarkers appear to surpass several traditional circulating markers in both predictive and diagnostic accuracy. For instance, EV-bound OPN, MMP-9 and GDF-15 have demonstrated superior sensitivity in discriminating between moderate and severe aortic stenosis when compared with plasma Fetuin-A or Gal-3 levels.[98,106–109] This superior predictive power is likely due to the compartmentalization and co-packaging of multiple bioactive molecules within EVs, which preserve biological context and enhance signal specificity. In contrast, traditional soluble markers such as Lp(a), fetuin-A or single cytokines often reflect systemic processes that are not valve-specific and may fluctuate in response to metabolic or inflammatory comorbidities.

Additionally, specific EV subpopulations, defined by markers such as Annexin V+ or CD144+ (endothelial marker), have been associated with a more rapid decline in hemodynamic stability exemplifying how understanding the specific origins of EVs can provide precise, personalized care.[110] These findings reinforce EVs as multidimensional biomarkers, capturing not only the presence but the biological stage and activity of CAVD. Despite their potential benefits, significant challenges remain before EVs can be fully integrated into clinical practice. Methodological heterogeneity in EV isolation, quantification and characterization hampers reproducibility across studies and limits cross-cohort validation.[106]

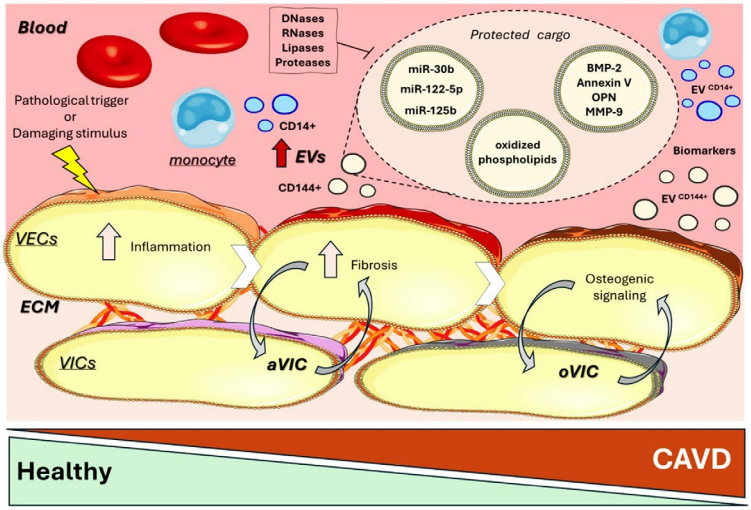

FIGURE 1 Schematic representation of extracellular vesicles as carriers of biomolecules relevant to the disease. Pathological triggers or damaging stimuli (e.g. mechanical stress, oxidative injury, inflammatory mediators) activate VECs, initiating inflammation and promoting extracellular matrix (ECM) remodelling. Activated valvular interstitial cells (aVIC) contribute to fibrosis, while osteogenic VICs (oVIC) drive osteogenic signalling and calcification. During these processes, VECs and immune cells (e.g. monocytes) release EVs into the bloodstream, including EV^{CD144+} (CD144 or VE-cadherin- endothelial marker) and EV^{CD14+} (CD14-monocyte marker).[104] These EVs encapsulate and protect molecular cargo—such as microRNAs (miR-30b, miR-122-5p, miR-125b), proteins such as bone morphogenetic protein-2 (BMP-2), Annexin V, osteopontin (OPN), matrix metalloproteinase-9 (MMP-9) and oxidized phospholipids (OxPL)—against degradation by circulating nucleases (DNases, RNases), lipases and proteases.[105] The protected cargo serves as potential diagnostic and prognostic biomarkers for CAVD, enabling detection at preclinical stages.

4 | STAGE-SPECIFIC BIOMARKER PROFILES

Taken together, in the early stages of disease progression, biomarkers of endothelial dysfunction and inflammation, including adhesion molecules (VCAM-1, ICAM-1), chemokines (CXCL12/CXCL6) and components of KLKB1 are prominent.[111,112] These markers may indicate a potentially reversible phase of the disease. Additionally, non-coding RNAs and cargo from EVs are also early indicators, capable of detecting subclinical molecular changes before structural valve calcification occurs.[113,114]

In contrast, proteins linked to osteogenic differentiation, such as BMP-2, OPN, ALP and OPG, are typically expressed in more advanced, irreversible stages of the disease, marking the onset of valvular calcification.[9,115] Thus, while endothelial, inflammatory and EV-derived signals dominate the early phase, mineralization-related proteins are more characteristic of the chronic, structural phase of CAVD (Table 1).

5 | CONCLUSIONS AND FUTURE DIRECTIONS

Despite the substantial expansion of candidate biomarkers in CAVD, their clinical translation remains constrained by heterogeneous performance and limited standardization.[98,116] In comparative terms, Lp(a) is optimal for risk stratification and genetic screening; endothelial and inflammatory markers (CXCL12, KLKB1, VCAM-1) are valuable for early disease detection; and EV-derived and osteogenic proteins (OPN, BMP-2, annexins) are superior for disease progression and prognosis. Conversely, systemic proteins such as Fetuin-A and Gal-3, while informative, exhibit reduced diagnostic specificity because they are modulated by comorbidities, including renal dysfunction or systemic fibrosis.[82] Thus, their actual value may lie in combination with imaging and clinical data.

A significant challenge is the lack of standardized, routine assays for both traditional circulating proteins and new EV-derived extracellular vesicle signatures, which impedes cross-study comparison.[117] Equally important is the necessity to connect biomarkers not only to structural changes in the valve but also to the underlying molecular activity that promotes inflammation, fibrosis and calcification.[104,105] From a translational standpoint, multiplexed platforms integrating proteomic, transcriptomic, lipidomic and EV-RNA readouts could better capture complementary disease pathways and improve risk stratification and therapeutic monitoring.[98,100–103] Such integrative panels could delineate distinct biological phenotypes inflammatory-active, fibrotic or calcific—by combining circulating and EV-based biomarkers with imaging modalities such as PET to detect microcalcification and CT

for calcium scoring. This systems-biology approach, supported by machine learning algorithms, may ultimately enable the transition from static, single-analyte testing to dynamic, precision biomarker profiling, guiding early diagnosis, therapeutic timing and monitoring of disease-modifying interventions in CAVD.

AUTHOR CONTRIBUTIONS

Alberto Cook-Calvete, Silvia Moreta, Maria Delgado-Marin and Blanca Fernandez-Rodriguez contributed substantially to the conception or design of the work, or the acquisition, analysis or interpretation of data for the work. Carlos Zaragoza and Marta Saura drafted the work and critically revised it for its intellectual content.

ACKNOWLEDGEMENTS

The authors have nothing to report.

FUNDING INFORMATION

This study was not funded by any grant.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data availability does not apply to this article as no new data were created or analysed in this study.

ORCID

Silvia Moreta https://orcid.org/0009-0002-1197-8330
Carlos Zaragoza https://orcid.org/0000-0002-1706-8592

REFERENCES

1. Kraler S, Blaser MC, Aikawa E, Camici GG, Lüscher TF. Calcific aortic valve disease: from molecular and cellular mechanisms to medical therapy. *Eur Heart J*. 2022;43(7):683-697.
2. Wang B, Mei Z, Yang H, Gao W, Ma L, An G. Global, regional, and national burden of nonrheumatic calcific aortic valve disease based on GBD study 2021. *Sci Rep*. 2025;15(1):29464.
3. Jain H, Goyal A, Khan ATMA, et al. Insights into calcific aortic valve stenosis: a comprehensive overview of the disease and advancing treatment strategies. *Ann Med Surg*. 2024;86(6):3577-3590.
4. Banovic M, Iung B, Wojakowski W, Van Mieghem N, Bartunek J. Asymptomatic Severe and Moderate Aortic Stenosis: Time for Appraisal of Treatment Indications. *Structural Heart*. 2023;7(5):100201.
5. Bhattacharyya S, Hayward C, Pepper J, Senior R. Risk stratification in asymptomatic severe aortic stenosis: a critical appraisal. *Eur Heart J*. 2012;33(19):2377-2387.
6. Lisi M, Cameli M, Mandoli GE, et al. Detection of myocardial fibrosis by speckle-tracking echocardiography: from prediction to clinical applications. *Heart Fail Rev*. 2022;27(5):1857-1867.
7. Moncla LHM, Briend M, Bossé Y, Mathieu P. Calcific aortic valve disease: mechanisms, prevention and treatment. *Nat Rev Cardiol*. 2023;20(8):546-559.
8. Mirzaalikhan Y, Lai A, Khanmohammadi M, et al. A disease-inspired in vitro model of aortic valve stenosis to investigate the drivers of endothelial–mesenchymal transition. *Lab Chip*. 2025;25(18):4635-4649.
9. Fan P, Liu Y, Qian X, et al. Deciphering the Enigma of Calcific Aortic Valve Disease: The Pivotal Role of Animal Models in Unraveling Pathogenesis and Advancing Therapeutic Strategies. *Biomedicine*. 2025;13(10):2369.
10. Wang Y, Osborne MT, Tung B, Li M, Li Y. Imaging Cardiovascular Calcification. *J Am Heart Assoc*. 2018;7(13):e008564.
11. Small AM, Yutzey KE, Binstadt BA, et al. Unravelling the mechanisms of valvular heart disease to identify medical therapy targets: a scientific statement from the american heart association. *Circulation*. 2024;150(6):e109-e128.
12. Small A, Kiss D, Giri J, et al. Biomarkers of Calcific Aortic Valve Disease. *Arterioscler Thromb Vasc Biol*. 2017;37(4):623-632.
13. Drăgan A, Mateescu AD. Novel biomarkers and advanced cardiac imaging in aortic stenosis: old and new. *Biomolecules*. 2023;13(11):1661.
14. Shelbaya K, Arthur V, Yang Y, et al. Large-scale proteomics identifies novel biomarkers and circulating risk factors for aortic stenosis. *J Am Coll Cardiol*. 2024;83(5):577-591.
15. Farrar EJ, Huntley GD, Butcher J. Endothelial-derived oxidative stress drives myofibroblastic activation and calcification of the aortic valve. *PLoS One*. 2015;10(4):e0123257.
16. Liu X, Li T, Sun J, Wang Z. The role of endoplasmic reticulum stress in calcific aortic valve disease. *Can J Cardiol*. 2023;39(11):1571-1580.
17. Butcher JT, Tressel S, Johnson T, et al. Transcriptional profiles of valvular and vascular endothelial cells reveal phenotypic differences. *Arterioscler Thromb Vasc Biol*. 2006;26(1):69-77.
18. Su X, Ao L, Shi Y, Johnson TR, Fullerton DA, Meng X. Oxidized low density lipoprotein induces bone morphogenetic protein-2 in coronary artery endothelial cells via toll-like receptors 2 and 4. *J Biol Chem*. 2011;286(14):12213-12220.
19. Simmons CA. Aortic Valve Mechanics. *J Am Coll Cardiol*. 2009;53(16):1456-1458.
20. Bouchareb R, Mahmut A, Nsaibia MJ, et al. Autotaxin derived from lipoprotein(a) and valve interstitial cells promotes inflammation and mineralization of the aortic valve. *Circulation*. 2015;132(8):677-690.
21. Tanase DM, Valasciuc E, Gosav EM, et al. Contribution of oxidative stress (OS) in calcific aortic valve disease (CAVD): from pathophysiology to therapeutic targets. *Cells*. 2022;11(17):2663.
22. Xiao F, Zha Q, Zhang Q, et al. Decreased Glucagon-Like Peptide-1 Is Associated With Calcific Aortic Valve Disease: GLP-1 Suppresses the Calcification of Aortic Valve Interstitial Cells. *Front Cardiovasc Med*. 2021;26:8.
23. Xu K, Xie S, Huang Y, et al. Cell-type transcriptome atlas of human aortic valves reveals cell heterogeneity and endothelial to mesenchymal transition involved in calcific aortic valve disease. *Arterioscler Thromb Vasc Biol*. 2020;40(12):2910-2921.
24. Paranya G, Vineberg S, Dvorin E, et al. Aortic valve endothelial cells undergo transforming growth factor-β-mediated and non-transforming growth factor-β-mediated transdifferentiation in vitro. *Am J Pathol*. 2001;159(4):1335-1343.

25. The E, de Graaf DM, Zhai Y, et al. Interleukin 38 alleviates aortic valve calcification by inhibition of NLRP3. *Proc Natl Acad Sci*. 2022;119(36):e2202577119.
26. Lu J, Xie S, Deng Y, Xie X, Liu Y. Blocking the NLRP3 inflammasome reduces osteogenic calcification and M1 macrophage polarization in a mouse model of calcified aortic valve stenosis. *Atherosclerosis*. 2022;347:28-38.
27. Glasenapp A, Derlin K, Gutberlet M, et al. Molecular imaging of inflammation and fibrosis in pressure overload heart failure. *Circ Res*. 2021;129(3):369-382.
28. Guo X, Zhang J, Huang M, et al. Systemic inflammation is associated with myocardial fibrosis in patients with obstructive hypertrophic cardiomyopathy. *ESC Heart Fail*. 2025;12(1):582-591.
29. Choi B, Kim EY, Kim JE, et al. Evogliptin suppresses calcific aortic valve disease by attenuating inflammation, fibrosis, and calcification. *Cells*. 2021;10(1):57.
30. Acharya A, Hans CP, Koenig SN, et al. Inhibitory role of Notch1 in calcific aortic valve disease. *PLoS One*. 2011;6(11):e27743.
31. Fan L, Yao D, Fan Z, et al. Beyond VICs: Shedding light on the overlooked VECs in calcific aortic valve disease. *Biomed Pharmacother*. 2024;178:117143.
32. Rajamannan NM, Subramaniam M, Rickard D, et al. Human Aortic Valve Calcification is Associated with an Osteoblast Phenotype. *Circulation*. 2003;107(17):2181-2184.
33. Zhang C, Liu M, Wang X, et al. Mechanism of CircANKRD36 regulating cell heterogeneity and endothelial mesenchymal transition in aortic valve stromal cells by regulating miR-599 and TGF-β signaling pathway. *Int J Cardiol*. 2022;352:104-114.
34. Perrotta I, Sciangula A, Aquila S, Mazzulla S. Matrix Metalloproteinase-9 Expression in Calcified Human Aortic Valves. *Appl Immunohistochem Mol Morphol*. 2016;24(2):128-137.
35. Kaden JJ, Vocke DC, Fischer CS, et al. Expression and activity of matrix metalloproteinase-2 in calcific aortic stenosis. *Z Kardiol*. 2004;93(2):124-130.
36. Aikawa E, Nahrendorf M, Sosnovik D, et al. Multimodality Molecular Imaging Identifies Proteolytic and Osteogenic Activities in Early Aortic Valve Disease. *Circulation*. 2007;115(3):377-386.
37. Summerhill VI, Moschetta D, Orekhov AN, Poggio P, Myasoedova VA. Sex-specific features of calcific aortic valve disease. *Int J Mol Sci*. 2020;21(16):5620.
38. Santangelo G, Faggiano A, Bernardi N, Carugo S, Giammanco A, Faggiano P. Lipoprotein(a) and aortic valve stenosis: A casual or causal association? *Nutr Metab Cardiovasc Dis*. 2022;32(2):309-317.
39. Sastre-Oliva T, Corbacho-Alonso N, Albo-Escalona D, et al. The Influence of Coronary Artery Disease in the Development of Aortic Stenosis and the Importance of the Albumin Redox State. *Antioxidants*. 2022;11(2):317.
40. Aleksandric S, Banovic M, Beleslin B. Challenges in diagnosis and functional assessment of coronary artery disease in patients with severe aortic stenosis. *Front Cardiovasc Med*. 2022;9:849032.
41. Dweck MR, Jones C, Joshi NV, et al. Assessment of valvular calcification and inflammation by positron emission tomography in patients with aortic stenosis. *Circulation*. 2012;125(1):76-86.
42. Wu L, Huang K, Li Q, et al. Crosstalk between myofibroblasts and macrophages: A regulative factor of valvular fibrosis in calcific aortic valve disease. *Cell Biol Int*. 2023;47(4):754-767.
43. Klauzen P, Basovich L, Shishkova D, Markova V, Malashicheva A. Macrophages in calcific aortic valve disease: paracrine and juxtacrine disease drivers. *Biomolecules*. 2024;14(12):1547.
44. Ward LJ, Laucyte-Cibulskiene A, Hernandez L, et al. Coronary artery calcification and aortic valve calcification in patients with kidney failure: a sex-disaggregated study. *Biol Sex Differ*. 2023;14(1):48.
45. Yu X, Fu Z, Yu M, Shi Y. Association of lipoprotein(a) and LPA gene with calcific aortic valve disease. *Eur J Med Res*. 2025;30(1):787.
46. Lilly SM, Deshmukh AJ, Epstein AE, et al. 2020 ACC expert consensus decision pathway on management of conduction disturbances in patients undergoing transcatheter aortic valve replacement. *J Am Coll Cardiol*. 2020;76(20):2391-2411.
47. Thanassoulis G, Campbell CY, Owens DS, et al. Genetic associations with valvular calcification and aortic stenosis. *N Engl J Med*. 2013;368(6):503-512.
48. Jung JJ, Jadbabaie F, Sadeghi MM. Molecular imaging of calcific aortic valve disease. *J Nucl Cardiol*. 2018;25(4):1148-1155.
49. Leibundgut G, Scipione C, Yin H, et al. Determinants of binding of oxidized phospholipids on apolipoprotein (a) and lipoprotein (a). *J Lipid Res*. 2013;54(10):2815-2830.
50. Averna M, Cefalù AB. LP(a): The new marker of high cardiovascular risk. *Nutr Metab Cardiovasc Dis*. 2025;35(3):103845.
51. Arsenault BJ, Loganath K, Girard A, et al. Lipoprotein(a) and calcific aortic valve stenosis progression. *JAMA Cardiol*. 2024;9(9):835.
52. Bhatia HS, Zheng KH, Garg PK, et al. Lipoprotein(a) and aortic valve calcification. *JACC Cardiovasc Imaging*. 2023;16(2):258-260.
53. Makshood M, Joshi PH, Kanaya AM, et al. Lipoprotein (a) and aortic valve calcium in South Asians compared to other race/ethnic groups. *Atherosclerosis*. 2020;313:14-19.
54. Pantelidis P, Oikonomou E, Lampsas S, et al. Lipoprotein(a) and calcific aortic valve disease initiation and progression: a systematic review and meta-analysis. *Cardiovasc Res*. 2023;119(8):1641-1655.
55. Tsimikas S, Miyanohara A, Hartvigsen K, et al. Human oxidation-specific antibodies reduce foam cell formation and atherosclerosis progression. *J Am Coll Cardiol*. 2011;58(16):1715-1727.
56. Bortnick AE, Austin TR, Hamerton E, et al. Plasma proteomic assessment of calcific aortic valve disease in older adults. *J Am Heart Assoc*. 2025;14(5):e036336.
57. Myasoedova VA, Bertolini F, Valerio V, et al. The role of adiponectin and leptin in fibro-calcific aortic valve disease: a systematic review and meta-analysis. *Biomedicine*. 2024;12(9):1977.
58. Liu Y, Gu Y, Shen Y, et al. Association between serum leptin level and calcific aortic valve disease. *J Am Heart Assoc*. 2019;8(19):e012495.
59. Kraler S, Garg V, Akhmedov A. Calcific aortic valve disease: novel insights into nitric oxide signalling. *Eur Heart J*. 2022;43(17):1665-1667.
60. Sánchez-Esteban S, Castro-Pinto M, Cook-Calvete A, et al. Integrin-linked kinase expression in human valve endothelial cells plays a protective role in calcific aortic valve disease. *Antioxidants*. 2022;11(9):1736.
61. Herranz B, Marquez S, Guijarro B, et al. Integrin-linked kinase regulates vasomotor function by preventing endothelial nitric oxide synthase uncoupling. *Circ Res*. 2012;110(3):439-449.

62. Delgado-Marin M, Sánchez-Esteban S, Cook-Calvete A, Jorquera-Ortega S, Zaragoza C, Saura M. Indoxyl sulfate-induced valve endothelial cell endothelial-to-mesenchymal transition and calcification in an integrin-linked kinase-dependent manner. *Cells*. 2024;13(6):481.
63. Yue Y, Wang C, Benedict C, et al. Interleukin-10 deficiency alters endothelial progenitor cell-derived exosome reparative effect on myocardial repair via integrin-linked kinase enrichment. *Circ Res*. 2020;126(3):315-329.
64. Alrasheed NM, Alammari RB, Alshammari TK, et al. α1A Adrenoreceptor blockade attenuates myocardial infarction by modulating the integrin-linked kinase/TGF-β/Smad signaling pathways. *BMC Cardiovasc Disord*. 2023;23(1):153.
65. Lurins J, Lurina D, Svirskis S, et al. Impact of several pro-inflammatory and cell degradation factors in patients with aortic valve stenosis. *Exp Ther Med*. 2019;17:2433-2442.
66. Lin R, Zhu Y, Chen W, Wang Z, Wang Y, Du J. Identification of circulating inflammatory proteins associated with calcific aortic valve stenosis by multiplex analysis. *Cardiovasc Toxicol*. 2024;24(5):499-512.
67. Spinale FG. Epilysin (Matrix Metalloproteinase-28) joins the matrix metalloproteinase team on the field of postmyocardial infarction remodelling. *Circ Res*. 2013;112(4):579-582.
68. Zhou K, Guo T, Xu Y, Guo R. Correlation between plasma matrix metalloproteinase-28 levels and severity of calcific aortic valve stenosis. *Med Sci Monit*. 2020;26:e925260.
69. Sádaba JR, Martínez-Martínez E, Arrieta V, et al. Role for galectin-3 in calcific aortic valve stenosis. *J Am Heart Assoc*. 2016;5(11):e004360.
70. Luo J, Wang S, Liu X, et al. Galectin-3 promotes calcification of human aortic valve interstitial cells via the NF-kappa B signalling pathway. *Cardiovasc Diagn Ther*. 2022;12(2):196-207.
71. Bobrowska B, Wieczorek-Surdacka E, Kruszelnicka O, Chyrchel B, Surdacki A, Dudek D. Clinical correlates and prognostic value of plasma galectin-3 levels in degenerative aortic stenosis: a single-center prospective study of patients referred for invasive treatment. *Int J Mol Sci*. 2017;18(5):947.
72. Yu PJ, Skolnick A, Ferrari G, et al. Correlation between plasma osteopontin levels and aortic valve calcification: Potential insights into the pathogenesis of aortic valve calcification and stenosis. *J Thorac Cardiovasc Surg*. 2009;138(1):196-199.
73. Grau JB, Poggio P, Sainger R, et al. Analysis of osteopontin levels for the identification of asymptomatic patients with calcific aortic valve disease. *Ann Thorac Surg*. 2012;93(1):79-86.
74. Loudon BL, Ntatsaki E, Newsome S, et al. Osteoprotegerin and myocardial fibrosis in patients with aortic stenosis. *Sci Rep*. 2018;8(1):14550.
75. Adamczyk T, Mizia Stec K, Mizia M, et al. Biomarkers of calcification and atherosclerosis in patients with degenerative aortic stenosis in relation to concomitant coronary artery disease. *Pol Arch Intern Med*. 2012;122(1–2):14-21.
76. Ma T, Zhao J, Yan Y, et al. Plasma osteoprotegerin predicts adverse cardiovascular events in stable coronary artery disease: the PEACE trial. *Front Cardiovasc Med*. 2023;10:1178153.
77. Di Minno A, Zanobini M, Myasoedova VA, et al. Could circulating fetuin A be a biomarker of aortic valve stenosis? *Int J Cardiol*. 2017;249:426-430.
78. Larik MO. Fetuin-A levels in association with calcific aortic valve disease: A meta-analysis. *Atherosclerosis Plus*. 2023;54:27-29.
79. Koos R, Brandenburg V, Mahnken AH, et al. Association of fetuin-A levels with the progression of aortic valve calcification in non-dialyzed patients. *Eur Heart J*. 2009;30(16):2054-2061.
80. Lu D, Thum T. RNA-based diagnostic and therapeutic strategies for cardiovascular disease. *Nat Rev Cardiol*. 2019;16(11):661-674.
81. Badowski C, He B, Garmire LX. Blood-derived lncRNAs as biomarkers for cancer diagnosis: the Good, the Bad and the Beauty. *NPJ Precis Oncol*. 2022;6(1):40.
82. Shen Y, Li J, Zhao Z, Chen X. Progress on long non-coding RNAs in calcific aortic valve disease. *Front Cardiovasc Med*. 2025;12:1522544.
83. Guo Y, Li H, Huang B, Hou L. LncRNA HOTAIR ameliorates calcific aortic valve disease by sponging miR-29b. *Int J Cardiol*. 2024;408:132094.
84. Xie MY, Hou LJ. LncRNA MALAT1 aggravates calcific aortic valve disease by sponging miR-195. *Int J Cardiol*. 2021;333:161.
85. Wei J, Zhu X, Sun A, Yan X, Meng X, Ge S. Long non-coding RNA FGD5 antisense RNA 1 targets Baculovirus inhibitor 5 via microRNA-497-5p to alleviate calcific aortic valve disease. *Clin Hemorheol Microcirc*. 2024;86(3):285-302.
86. Song GY, Guo XN, Yao J, et al. Differential expression profiles and functional analysis of long non-coding RNAs in calcific aortic valve disease. *BMC Cardiovasc Disord*. 2023;23(1):326.
87. Yang R, Tang Y, Chen X, Yang Y. Telocytes-derived extracellular vesicles alleviate aortic valve calcification by carrying miR-30b. *ESC Heart Fail*. 2021;8(5):3935-3946.
88. Zhang F, Cheng N, Han Y, Zhang C, Zhang H. miRNA expression profiling uncovers a role of miR-139-5p in regulating the calcification of human aortic valve interstitial cells. *Front Genet*. 2021;12:722564.
89. Goody PR, Christmann D, Goody D, et al. Calcific aortic valve disease augments vesicular microRNA-145-5p to regulate the calcification of valvular interstitial cells via cellular crosstalk. *Basic Res Cardiol*. 2025;120:991-1010.
90. Ohukainen P, Syväranta S, Näpänkangas J, et al. MicroRNA-125b and chemokine CCL4 expression are associated with calcific aortic valve disease. *Ann Med*. 2015;47(5):423-429.
91. Oury C, Servais L, Bouznad N, Hego A, Nchimi A, Lancellotti P. MicroRNAs in valvular heart diseases: potential role as markers and actors of valvular and cardiac remodelling. *Int J Mol Sci*. 2016;17(7):1120.
92. Huber J, Longaker MT, Quarto N. Circulating and extracellular vesicle-derived microRNAs as biomarkers in bone-related diseases. *Front Endocrinol (Lausanne)*. 2023;24:1168898.
93. Krauze A, Procyk G, Gąsecka A, Garstka-Pacak I, Wrzosek M. The Role of MicroRNAs in Aortic Stenosis—Lessons from Recent Clinical Research Studies. *Int J Mol Sci*. 2023;24(17):13095.
94. Heuschkel MA, Skenteris NT, Hutcheson JD, et al. Integrative multi-omics analysis in calcific aortic valve disease reveals a link to the formation of amyloid-like deposits. *Cells*. 2020;9(10):2164.
95. Ragni E. Extracellular vesicles: recent advances and perspectives. *Front Biosci*. 2025;30(6):36405.
96. Jin Y, Chen K, Wang Z, et al. DNA in serum extracellular vesicles is stable under different storage conditions. *BMC Cancer*. 2016;16(1):753.
97. Ge Q, Zhou Y, Lu J, Bai Y, Xie X, Lu Z. miRNA in plasma exosome is stable under different storage conditions. *Molecules*. 2014;19(2):1568-1575.

98. Blaser MC, Buffolo F, Halu A, et al. Multiomics of tissue extracellular vesicles identifies unique modulators of atherosclerosis and calcific aortic valve stenosis. *Circulation*. 2023;148(8):661-678.
99. Martin PJ, Haren N, Ghali O, et al. Adipogenic RNAs are transferred in osteoblasts via bone marrow adipocyte-derived extracellular vesicles (EVs). *BMC Cell Biol*. 2015;16(1):10.
100. Resch C, Stamenkovic A, Surendran A, et al. Valvular oxidized phospholipids correlate with the severity of human aortic valvular stenosis. *Free Radic Biol Med*. 2025;239:219-229.
101. Xiao Y, Liang J, Witwer KW, Zhang Y, Wang Q, Yin H. Extracellular vesicle-associated microRNA-30b-5p activates macrophages through the SIRT1/NF-κB pathway in cell senescence. *Front Immunol*. 2022;13:955175.
102. Ferreira CR, Carpenter TO, Braddock DT. ENPP1 in blood and bone: skeletal and soft tissue diseases induced by ENPP1 deficiency. *Ann Rev Pathol Mechan Dis*. 2024;19(1):507-540.
103. Lorite P, Domínguez JN, Palomeque T, Torres MI. Extracellular vesicles: advanced tools for disease diagnosis, monitoring, and therapies. *Int J Mol Sci*. 2024;26(1):189.
104. Krohn JB, Aikawa E, Aikawa M, Hutcheson JD, Sahoo S, Fish JE. Editorial: Extracellular vesicles in cardiovascular inflammation and calcification. *Front Cardiovasc Med*. 2022;9:1077124.
105. Blaser MC, Kraler S, Lüscher TF, Aikawa E. Multi-omics approaches to define calcific aortic valve disease pathogenesis. *Circ Res*. 2021;128(9):1371-1397.
106. Diehl P, Nagy F, Sossong V, et al. Increased levels of circulating microparticles in patients with severe aortic valve stenosis. *Thromb Haemost*. 2008;99(4):711-719.
107. Hulanicka M, Garncarz M, Parzeniecka-Jaworska M, Jank M. Plasma miRNAs as potential biomarkers of chronic degenerative valvular disease in Dachshunds. *BMC Vet Res*. 2014;10(1):205.
108. Verwer MC, Mekke J, Timmerman N, et al. Comparison of cardiovascular biomarker expression in extracellular vesicles, plasma and carotid plaque for the prediction of MACE in CEA patients. *Sci Rep*. 2023;13(1):1010.
109. Jung RG, Duchez AC, Simard T, et al. Plasminogen activator inhibitor-1–positive platelet-derived extracellular vesicles predict MACE and the pro-inflammatory SMC phenotype. *JACC Basic Transl Sci*. 2022;7(10):985-997.
110. Sinning JM, Losch J, Walenta K, Bohm M, Nickenig G, Werner N. Circulating CD31+/Annexin V+ microparticles correlate with cardiovascular outcomes. *Eur Heart J*. 2011;32(16):2034-2041.
111. Troncoso MF, Ortiz-Quintero J, Garrido-Moreno V, et al. VCAM-1 as a predictive biomarker in cardiovascular disease. *Biochim Biophys Acta Mol Basis Dis*. 2021;1867(9):166170.
112. Wang D, Xiong T, Yu W, et al. Predicting the key genes involved in aortic valve calcification through integrated bioinformatics analysis. *Front Genet*. 2021;12:650213.
113. Zheng Y, Wen S, Jiang S, et al. CircRNA/lncRNA–miRNA–mRNA network and gene landscape in calcific aortic valve disease. *BMC Genomics*. 2023;24(1):419.
114. Liang R, Abudurexiti N, Wu J, et al. Exosomes and miRNAs in cardiovascular diseases and transcatheter pulmonary valve replacement: advancements, gaps and perspectives. *Int J Mol Sci*. 2024;25(24):13686.
115. Chen L, Liu H, Sun C, et al. A novel LncRNA SNHG3 promotes osteoblast differentiation through BMP2 upregulation in aortic valve calcification. *JACC Basic Transl Sci*. 2022;7(9):899-914.
116. Schlotter F, Halu A, Goto S, et al. Spatiotemporal multiomics mapping generates a molecular atlas of the aortic valve and reveals networks driving disease. *Circulation*. 2018;138(4):377-393.
117. Ong JYS, Tan SML, Koh AS, et al. Novel circulating biomarkers in aortic valve stenosis. *Int J Mol Sci*. 2025;26(5):1902.

How to cite this article: Cook-Calvete A, Moreta S, Delgado-Marin M, Fernandez-Rodriguez B, Zaragoza C, Saura M. Integrated biomarker landscape for the early detection and management of calcific aortic valve disease. *Eur J Clin Invest*. 2025;00:e70147. doi:10.1111/eci.70147